CLAIMS

What is claimed is:

1. A method for identifying compounds that affect LDL-proteoglycan binding, comprising the steps of:

(a) incubating a mixture comprising (i) proteoglycan, (ii) LDL, and (iii) a candidate compound, under conditions wherein LDL binds to proteoglycan to form an LDL-proteoglycan complex in the absence of said candidate compound;

(b) determining any difference between the amount of LDL-proteoglycan complex present in:

(i) the mixture prepared in step (a), and

(ii) a assay mixture comprising said proteoglycan and said LDL in the absence of said candidate compound.

2. The method according to claim 1, further comprising the step of

(c) correlating any difference determined in step (b) with said candidate compound's ability to affect LDL-proteoglycan binding.

3. The method according to claim 1 or 2, wherein the LDL of step (a) is attached to a solid support.

4. The method according to claim 1 or 2, wherein the proteoglycan of step (a) is attached to a solid support.

5. The method according to claim 1, 2 or 4, wherein the LDL of step (a) is labeled.

6. The method according to claim 3, wherein the proteoglycan of step (a) is labeled.

7. The method according to claim 6, wherein the label is biotin.

8. The method according to claim 7, further comprising the steps of:

contacting the solid support after the preparation of the assay mixture of step (a) with streptavidin peroxidase under conditions wherein biotin binds to streptavidin to form a biotin-avidin complex;

detecting any enzyme activity of the peroxidase bound to the solid support.

9. The method according to claim 1 or 2, wherein the proteoglycan of step (a) is labeled.

10. A method for identifying compounds which affect LDL-proteoglycan binding, which do not substantially affect LDL receptor binding, according to claims 2, further comprising the steps of:

(d) incubating a mixture comprising (i) LDL receptor, (ii) LDL, and (iii) a candidate compound that affects LDL-proteoglycan binding identified in step (c), under conditions wherein LDL binds to LDL receptor to form an LDL-LDL receptor complex in the absence of said inhibitor of LDL-proteoglycan binding;

(e) determining any difference between the amount of LDL-LDL receptor complex present in:

(i) the mixture prepared in step (d), and

(ii) a control mixture comprising said LDL receptor and said LDL in the absence of said inhibitor of LDL-proteoglycan binding.

11. The method according to claim 11, further comprising the step of:

(f) correlating any difference determined in step (e) with said candidate compound's ability to affect LDL-LDL receptor binding activity.

12. The compounds that affect LDL-proteoglycan binding identified by the method according to claim 1 or 2.

13. The compounds which affect LDL-proteoglycan binding, which do not substantially affect LDL receptor binding identified by the method according to claim 10 or 11.

14. A apo-B100 protein comprising a proteoglycan$^-$receptor$^+$ mutation in Site B.

15. The apo-B100 protein according to claim 14, which is purified.

16. The apo-B100 protein according to claim 14, which is synthesized by recombinant DNA expression or chemical synthesis.

17. The apo-B100 protein according to claim 14, wherein the amino acid sequence from position 3358 to 3359 is selected from the group consisting of:

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-**Glu_{3363}**-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367},

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-**Asp_{3363}**-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367},

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-**Ala_{3363}**-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367}

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-**Thr_{3363}**-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367}

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-**Ser_{3363}**-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367}

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-**Gln_{3363}**-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367}

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-**Glu_{3362}**-Lys_{3363}-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367},

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-**Asp_{3362}**-Lys_{3363}-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367},

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-Lys_{3363}-**Glu_{3364}**-Gly_{3365}-Leu_{3366}-Lys_{3367},

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-Lys_{3363}-**Asp_{3364}**-Gly_{3365}-Leu_{3366}-Lys_{3367},

Thr_{3358}-**Glu_{3359}**-Leu_{3360}-Thr_{3361}-Arg_{3362}-Lys_{3363}-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367},

Thr_{3358}-**Asp_{3359}**-Leu_{3360}-Thr_{3361}-Arg_{3362}-Lys_{3363}-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367},

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}----------Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367},

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}----------Lys_{3363}-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367},

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-Lys_{3363}----------Gly_{3365}-Leu_{3366}-Lys_{3367},

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-**Glu**-Lys_{3363}-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367},

Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-Lys_{3363}-**Glu**-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367},
Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-**Asp**-Lys_{3363}-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367}, and
Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-Lys_{3363}-**Asp**-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367}.

18. The apo-B100 protein according to claim 14, wherein said mutation in Site B is the K3363E mutation, and the amino acid sequence from position 3358 to 3359 is:
Thr_{3358}-Arg_{3359}-Leu_{3360}-Thr_{3361}-Arg_{3362}-**Glu_{3363}**-Arg_{3364}-Gly_{3365}-Leu_{3366}-Lys_{3367}.

19. A polypeptide comprising the amino acid sequence of Site B in the apo-B100 protein according to any one of claims 14 to 18, wherein said Site B is flanked on at least one side by a contiguous sequence of at least 10 amino acids which is directly adjacent to Site B in the wild-type human apo-B100 sequence.

20. An LDL particle comprising an apo-B100 protein according to any one of claims 14 to 18.

21. An LDL particle comprising a polypeptide according to claim 19.

22. An antibody composition which binds to an antigenic determinant in an apo-B100 protein according to any one of claims 14 to 18, wherein said antigenic determinant is not present in the wild-type human apo-B100 protein.

23. A polynucleotide encoding an apo-B100 protein according to any one of claims 14 to 18.

24. The polynucleotide according to claim 23, wherein the polynucleotide is present in a 95-kb apo-B P1 plasmid p158.

25. The polynucleotide according to claim 24, wherein said mutation in Site B is the K3363E mutation.

26. A cell comprising polynucleotide according to any one of claims 23 to 25.

27. A non-human animal comprising a polynucleotide according to any one of claims 23 to 25.

28. A method for preventing or reducing the severity of atherosclerosis in a animal, comprising expressing a polynucleotide according to any one of claims 23 to 25.